REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
24-12-2012	Final Report	5-Sep-2009 - 4-Sep-2012

4. TITLE AND SUBTITLE
Potentiometric Detection of Pathogens

5a. CONTRACT NUMBER
W911NF-09-1-0499

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHORS
Kalle Levon

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
Polytechnic University - Brooklyn
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
56787-CH.4

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The project started with a goal of developing microfluidic single working electrode device using open circuit potentiometry as the transduction method. The uniqueness was based on the high surface area of the nanosize organic electrode (conducting polymer top-layer) surface. This approach has then been changed to the gate modification in ion sensitive field effect transistors, in which our unique approach contains floating gate approach to make the operation more simple. Presently our FGFET-arrays (floating gate ion sensitive field effect transistor

15. SUBJECT TERMS
Ion sensitive Transistor, Immunoassay

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Kalle Levon
UU	UU	UU	UU		19b. TELEPHONE NUMBER 718-260-3339

Report Title

Potentiometric Detection of Pathogens

ABSTRACT

The project started with a goal of developing microfluidic single working electrode device using open circuit potentiometry as the transduction method. The uniqueness was based on the high surface area of the nanosize organic electrode (conducting polymer top-layer) surface. This approach has then been changed to the gate modification in ion sensitive field effect transistors, in which our unique approach contains floating gate approach to make the operation more simple. Presently our FGFET-arrays (floating gate ion sensitive field effect transistor arrays)consist of encapsulated 8x8 (64) gate arrays, and the next designs have been completed for 16x64 and 64x64 arrays to monitor changes in analytes with a large number of proteins. Antigen binding is monitored using capture antibody or capture imprint and both validated with monoclonal antibody conjugated with HRP enzyme which acts as a charge-amplifier.

The final project report describes first the development of the conducting polymer top-layer, which makes the devices very functional and competitive. Secondly, the device development is discussed and finally the preclinical results are presented with a main focus on the recent development of cardiac marker panel with five different markers.

As the use of semiconductor technology for DNA sequencing has induced a major change in the field of sequencing, we pioneer similar trends for protein-protein interaction studies. Continuous monitoring of over one thousand protein-protein interactions will yield novel information of disease progression especially in its early stages.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
08/30/2011	2.00	Jeannine Larrieux, Osvaldo Abrou, Kalle Levon. Potentiometric Detection of K+ Ions Using Gramicidin-Ionophore/Lipid Bilayer Assembly on Polyaniline Electrode, Macromolecular Symposia, (06 2011): 0. doi: 10.1002/masy.201150602
TOTAL:	**1**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
08/30/2011	3.00	. Morphological and Spectroscopic Studies of ElectrochemicallyDeposited Poly(3,4-ethylenedioxythiophene) (PEDOT) HoleExtraction Layer for Organic Photovoltaic Device (OPVd) Fabrication, (02 2011)

TOTAL: **1**

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

FDiPro award 2009-2014
Bioaccelarate Award 2010
NYU Innovention Award 2011

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Alok Prabhu	1.00	
Eduard Nasybulin	1.00	
FTE Equivalent:	**2.00**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Yanyan Wang	1.00
Vladimir Egorov	1.00
FTE Equivalent:	**2.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Kalle Levon	0.10	
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Joseph Asad	0.00	Bsc
samantha Murray	0.00	Bsc
Jessica Yee	0.00	Bsc
FTE Equivalent:	**0.00**	
Total Number:	**3**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	6.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	6.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	4.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	2.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	2.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Aabhas Martin
Hari Prasad
Total Number: 2

Names of personnel receiving PHDs

NAME
Alok Prabhu
Eduard Nasybulin
Qi Zhang
Total Number: 3

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

FINAL PROJECT REPORT

The project started with a goal of developing microfluidic single working electrode device using open circuit potentiometry as the transduction method. The uniqueness was based on the high surface area of the nanosize organic electrode (conducting polymer top-layer) surface. This approach has then been changed to the gate modification in ion sensitive field effect transistors, in which our unique approach contains floating gate approach to make the operation more simple. Presently our FGFET-arrays (floating gate ion sensitive field effect transistor arrays)consist of encapsulated 8x8 (64) gate arrays, and the next designs have been completed for 16x64 and 64x64 arrays to monitor changes in analytes with a large number of proteins. Antigen binding is monitored using capture antibody or capture imprint and both validated with monoclonal antibody conjugated with HRP enzyme which acts as a charge-amplifier.

The final project report describes first the development of the conducting polymer top-layer, which makes the devices very functional and competitive. Secondly, the device development is discussed and finally the preclinical results are presented with a main focus on the recent development of cardiac marker panel with five different markers.

As the use of semiconductor technology for DNA sequencing has induced a major change in the field of sequencing, we pioneer similar trends for protein-protein interaction studies. Continuous monitoring of over one thousand protein-protein interactions will yield novel information of disease progression especially in its early stages.

OUTLINE

1. Surface modification
1.1. Organic conducting layer
1.1.1. Surface area maximization: Nanofibers from interfacial polymerization
1.1.2. Precision: WF control with oligomers
1.1.3. Buffer stability: selection of counter ions for the polarons
1.2. Immobilization: Glutaldehyde (GA) conjugation
1.3. Prevention of non-specific binding - GA conjugation of BSA
2. Device development
2.1. Flow injection analysis – Screen Printed Electrodes
2.2. Floating gate ion sensitive field effect array
3. Preclinical Studies
3.1. Cancer Patient Breath Analysis (40 ppb LOD)
3.2. Cell Based Screening
3.3. DNA label free SNP Analysis
3.4. Antigen detection
3.4.1. Capture Ab for ITC complex
3.4.2. Imprinting: CEA, HAPLN1

1. SURFACE MODIFICATION

1.2. Organic conducting layer

1.1.1. Nanofibers from interfacial polymerization

Electrochemical Interfacial polymerization (in acetonitrile/toluene) was conducted with PEDOT. The impact of the Nan fiber morphology was evidenced first by the unique performance of the hole transport layer. Presently the same method, but in a chronopotentiometric format is used for the preparation of polyaniline nanofibers on electrode surfaces for enhanced surface area in diagnostics applications.

Ref: Morphological and Spectroscopic Studies of Electrochemically Deposited Poly(3,4-ethylenedioxythiophene) (PEDOT) Hole Extraction Layer for Organic Photovoltaic Device (OPVd) Fabrication, E. Nasybulin, S. Wei, M. Cox, I. Kymissis, K. Levon, J Phys Chem C, 2011, 115, 4307-4314

1.1.2. Precision: WF control with oligomers

Polyaniline is a unique semiconductor as the doping process involves only protonation/deprotonation steps. Neither oxidation nor reduction is needed for the sensitive ion exchange phenomenon. Our manuscript presents the control parameters using electroactive oligomeric models.

Intramolecular Transport of Charge Carriers in Trimeric Aniline upon a Three-Step Acid Doping Process, Zhang, Q., Khajo, A., Sai, T., de Albuquerque, I., Magliozzo, R.S., Levon K., J Phys Chem A 116, 7629 – 7635 (2012)

1.1.3. Buffer stability: selection of counter ion

We have found out that macromolecular counter ions create pH stability at PBS pH 7.4 solutions. The stability accounts for the stability between two macromolecules (Interpolymer complex between polyaniline and macromolecular counter ions and the entanglements involved with the macromolecules). Sulfonated polystyrene, polyvinylphosphate, polyalkyldiaminechloride (PDMAC) and polyethylene imine (PEI) were used. PEI caused the deprotonation regardless of the nature of the electrolyte. Similarly hydrophobic counter ions such as dinonylnapthalene and dibenzyl sulfonic acids, prevented the effect of a strong base deprotonating the complexes but also prevented any other binding with polar molecules.

Thus we have developed polyaniline based systems which are stable in buffer but still sensitive to macromolecular binding events. As an example: Potentiometric Detection of DNA Hybridization, Zhou, J., Yu, B., Sergeyev, V., Giuseppi-Elie, A., Levon, K., Biosens. Bioelectron. 24 (2009) 3275–3280

1.2. Immobilization

Immobilization: in the beginning of the project we applied thiolation of polyaniline as the immobilization but due to too extensive reduction of polyaniline, we have changed to the use of glutaldehyde (GA), which first binds to the secondary nitrogen in the polyaniline surface and then the remaining aldehydes react first with the capture antibodies and then with BSA.

1.3. Prevention of non-specific binding

Initially thiolation was applied also for the build-up of the prevention layer but due to the excessive reduction of the conducting polymer, GA conjugation was taken as the main method. GA first binds to the secondary nitrogen in the polyaniline surface and then the remaining aldehydes react first with the capture antibodies and then with BSA.

2. DEVICE DEVELOPMENT

2.1. ICP Flow injection analysis (ICPFA)– Screen Printed Electrodes

FIA system with screen printed electrodes were purchased from DropSens Inc. This system was applied for the breath analysis system as well as for the analysis of all conducting polymer based “single electrode” evaluations. The flow analysis system is also used for the selection of all the control parameters for the immunoassaying.

Annexin A2 binds to endosomes following organelle destabilization by particulate wear debris, Scharf, B., Clement, C.C., Wu, X.-X., Morozova, K., Zanolini, D., Follenzi, A., Larocca, J.N., Levon, K., Sutterwala, F.S., Rand, J., Cobelli, N., Purdue, E., Hajjar, K.A., Santambrogio, L., Nature Communications, 2012, 3 1-10

Ionophore/Lipid Bilayer Assembly on Soft Organic Electrodes for Potentiometric Detection of K+ Ions, O. Abreu. J. Larrieux, K. Levon, Ch 14 in Aspects on Fundaments and Applications of Conducting Polymers Ed. A. de Jesus Motheo InTechOpen (2012) 67-86

Potentiometric Detection of K+ Ions Using Gramicidin-Ionophore/Lipid Bilayer Assembly on Polyaniline electrode, O. Abreu, J. Larrieux, K. Levon, Makromol. Symp. 2011, 304, 8-17

2.2. Floating gate ion sensitive field effect array

50 FGFETs were designed and the manufacturing was ordered from MOSIS. The chips had electrode designs for individual gate and 2x2 and 8x8 gate arrays. Electrical validation along with testing conditions (temperature, humidity) were done first. Further the encapsulation of the detection area was done with SU8 technology and the chips have been used in an in-out fluidic mode (same, single analyte solution). The chips have also been compared in floating gate vs reference electrode modes. Leakage conditions have also been determined. The target analyses have been pH, polyelectrolytes and selected proteins.

3. Preclinical Studies

3.1. Cancer patient breath analysis

The project has two components: first the establishment of the gas calibration curves using gas dilution system. Ammonium (NH_3) was diluted in three different gases and detection limit was shown to be 20 ppb.

The second part entailed testing of 40 patient samples, cancer patients being blinds samples. The two positive samples were detected quantitatively with one false positive sample. The detection limit with the positive samples was 40 ppb.

3.2.Cytotoxicity measurement by potentiometry

The profile of attachment in Figure shows adequate stabilization after addition of the buffer medium. The first two peaks show the rise and fall back of the potential after adding the medium which implies the contribution of the medium towards potential change is not very significant after a time of 20-40 min. Hence significant potential difference between the electrodes is due to cells.

The profile of Triton-x cytotoxicity in Figure shows the decrease in negative potential after addition of Triton-x to the monolayer of cells, in the well, at t=0. This is due to cell death as a response to Triton-x. The results are very promising and in the future, ion selective electrodes will be applied.

3.3. DNA label free SNP Analysis

The main problem has been the fast deprotonation of doped polyaniline in buffer. Although we have developed several doped polyaniline systems which remain electroactive in buffer, (self-doping, hydrophobic counter-ions, macromolecular counter ions as examples), the ion exchange process still is a non-equilibrium process. We have solved the problem by relying on a macromolecular interpolymer complex formation: oligomeric single strand DNA samples replaces the counter-ion and provides stability against deprotonation caused by buffer. We now can follow the SNP detection quantitatively in a completely label-free process. We have now initial results that our charge-based detection system is able to identify sheared genomic samples thus proving that PCR-based amplification is not needed.

Potentiometric Detection of DNA Hybridization, Zhou, J., Yu, B., Sergeyev, V., Giuseppi-Elie, A., Levon, K., Biosens. Bioelectron. 24 (2009) 3275–3280

Potentiometric Monitoring DNA Hybridization with Polyaniline/Nylon-6 Working Electrode, Kalle Levon, Eduard Nasybulin, Irina Menshikova, Vladimir Sergeyev, Alexander Zezin, Polymer Science, Ser. A, 2009, Vol. 51, No. 6, pp. 701–707

3.4. Ab-Ag with ITC complex

3.4.1. Amplification

Commercial monoclonal antibodies conjugated with Horse Radish Peroxidase enzymes (mAb-HRP) have been applied to the detection of ITC (troponin complex), important in cardiac panels. The panel includes testing of five cardiac markers. The testing was one with the developed polyaniline chip and will next be applied to FGFET array. The testing limit presently was 3 orders of magnitude better than with a commercial ELISA test.

In the main approach, either with a ICP flow analysis or with FGISFET array, we monitor the impact of changed charge during the biological binding. Recently, our biggest success has been the application of charge amplification. Thus in addition to monitoring the capture antibody binding with the antigen, we introduce monoclonal antibody, which confirms the selectivity of the binding, conjugated with HRP enzyme. The enzymatic redox reaction influences the OCP or the threshold voltage (Vth) significantly. With the ICP flow analysis, we have shown that the LOD is 2 orders of magnitude better than with a commercial ELISA test.

3.4.2. Imprinting: CEA, HAPLN1

Carcinoembryonic antigen (CEA) was imprinted in the early stage of the project. Two publications present the technology. CEA capture imprinting results showed linear validation with standard Elisa steps in serum. Presently human patients samples are being validated and the early result with 9 positive responses presents similar linear validation with Elisa test. The capture capability is very selective but the binding can be validated using MAb's.

Yantian Wang, Zhiquan Zhang, Vijay Jain, Jinju Yi, Steffen Mueller, Jonathan Sokolov,
Zhenxian Liuf, Kalle Levon, Basil Rigas, Miriam H. Rafailovich 'Potentiometric sensors based on surface molecular imprinting: Detection of cancer biomarkers and viruses' Sensors and Actuators B 146 (2010) 381–387

HAPLN1 protein is over expressed in the majority of mesotheliomas and hence being considered as a potential biomarker for cancer detection. In a clean glass container 20 µg of HAPLN1 (65.04 kDa) protein was dissolved in 10 ml of de-ionized water (i.e 0.03 µM) and 4 mg 11 mercapto 1-undecanol was dissolved in 10 ml ethanol (i.e 0.1 mM). A mixed solution is formed by dissolving the two in 19:1 ratio (water: ethanol) such that the final concentration of thiol in the solution becomes 0.1mM. Three gold electrodes A, B and C were dipped in the solution for 12 hours and then the electrodes were rinsed thoroughly with de-ionized water to remove bound protein on the surface.

Similarly HAPLN1 electrodes were also checked for potentiometric response to the cancer biomarker spiked in serum. To prepare 6.5µg/ml (100 nM) standard spike solution, 100 µl of human serum was diluted with 650 µl of 1X PBS solution and 250 µl of (25.5 µg of HAPLN1). Since 10 µl of solution was used for titration in 10 ml PBS buffer, the highest concentration of protein is 10-10 M. Remaining standards were prepared by maintaining the same dilution rate (1:8) of serum in buffer but analyte concentration was reduced ten times with serial dilutions from the stock solution.

HAPLN1 imprinting results have been accepted to be published in PLOS One with reviewers' comments stating that the results are disruptive with clinical relevance.
Aabhas Mathur, Steven Blias, Thomas Neubert, Harvey Pass and Kalle Levon, DEVELOPMENT OF BIOSENSOR FOR DETECTION OF PLEURAL MESOTHELIOMA CANCER BIOMARKER USING IMPRINTED HYDROXYLATED ALKANETHIOLS ON GOLD SURFACE, PLOS One accepted.

Technology Transfer